CHAPTER **18**

AUTOLOGOUS BONE MARROW-DERIVED STEM CELLS FOR CHRONIC WOUNDS OF THE LOWER EXTREMITY: A RETROSPECTIVE STUDY

Nilofar Faghihnia, DPM
Gerit D. Mulder, DPM
Daniel K. Lee, DPM

INTRODUCTION

Chronic lower extremity ulcers are a physical and financial burden to the health and economic establishment in the US and Worldwide. Lower extremity wounds occur in 4-10% of people with diabetes, with a lifetime risk of up to 25%, and a 20-80% recurrence rate. Foot complications account for about 25% of all diabetic admissions with 100,000 lower-limb amputations performed annually (1). The cost of treating an ulcer may range up to $48,000 per year not including the cost of the amputation or any secondary problems. Early detection and appropriate treatment of these ulcers may prevent up to 85% of amputations. Most lower extremity wounds are caused by venous disease, arterial insufficiency, diabetic neuropathy, or a combination of these factors (2). Other etiologies may include vasculitis, pressure, dehisced surgical incisions, burns, and trauma. Venous leg ulcers affect up to 1% of the world's population, account for almost 70% of all chronic leg wounds with an estimated cost to heal of $6,449 per year (2-6). Recent evidence suggests that stem cells derived from bone marrow have potential to treat many disorders given their plasticity and ability to differentiate into various types of tissues, including skin cells (7-12).

The primary purpose of expediting wound closure in chronic and problematic lower extremity wounds is to reduce the risk of amputation as well as associated co-morbidities including infection and tissue necrosis. Additional care and cost include but are not limited to the administration of antibiotics, temporary off-loading devices (temporary shoes, contact casts, walkers), hospitalization, and surgery. Also, there are no financial amounts that can be associated with the mental and physical effects on the individual's lifestyle, decrease in quality of life, and effect on society in general. Expediting closure of lower extremity wounds while decreasing associated complications would have a significant impact on quality of life, rate of morbidity and mortality, and cost of care associated with this population.

There are several factors that contribute to delayed wound healing at the host level including macrovascular disease, hyperglycemia, and increased venous pressure. In chronic wounds, the senescent cells due to the inhibition of fibroblast proliferation are unable to divide and become unresponsive to growth factors. Stanley and Osler showed that a human venous leg ulcer with more than 15% of senescent cells would be more difficult to heal (13).

Application of stem cells in the bone marrow aspirate (BMA) locally to the wound bed has shown promising results for treatment of lower extremity ulcers. These stem cells are easily derived from the patient's own bone marrow, eliminating the risk of transmission of infectious disease transmission with allogeneic products. The aspirates contain 2 types of stem cells: hematopoietic and mesenchymal. The hematopoietic stem cells differentiate into red and white blood cells, platelets, and macrophages. The mesenchymal stem cells are multipotent and differentiate into multiple cell types involved in tissue repair, when placed in the appropriate microenvironment. (14)

Bone marrow aspirates consist of inflammatory cell progenitors, which have been shown to participate in wound healing, mesenchymal stem cells, which appear to be phynotypically altered and/or senescent in chronic wounds, and multipotent stem cells (15-19). The progenitor cells show great potential in healing chronic wounds due to their unique immunologic properties and regenerative potential. However, questions still remain regarding the clinical mechanism of cell migration and proliferation, and of extracellular matrix deposition and remodeling after application of BMA derived stem cells. The most significant problem with bone marrow aspirate use to date is the inability to quantify the number of viable stem cells once the BMA is extracted and immediately put on the wound. Cellular infiltration and regeneration in chronic wounds is poorly defined. Cellular senescence and the presence of biofilm in the wound bed are also important considerations as they create barriers to healing in the chronic wound. When not adequately addressed,

the latter factors are known to impede wound closure and may also prevent effective stem cell activity.

Current treatments and traditional approaches to wound closure, including thorough debridement, have had limited success and do not appear to have significantly decreased amputation rates in patients with underlying deficiency of required cellular activity. While debridement is successful in removing inhibitors and barriers to effective wound closure, including senescent cells, nonviable tissue and bacteria harbored therein, it does not address the inability of cells in select populations to replicate.

This retrospective review was designed to help determine the potential efficacy and use of BMA derived stem cells in chronic wounds of the lower extremity of multiple etiologies that had failed all other forms of treatment. The goal of the review was to determine if autologous bone marrow stem cell aspirate had the potential to assist non-healing lower extremity ulcers that have been unresponsive to traditional methods by expediting wound closure and preventing further need for surgical intervention. The limitations, which are well recognized by the authors, are the relatively small population reviewed, including numbers and etiology as well as lack of randomization. However, the purpose of this review is similar to a Phase I trial in determining support for a concept versus demonstrating clinical efficacy. It is not the intent of the paper to discredit potential future use of BMA.

MATERIALS AND METHODS

Patient Selection

Patients were selected from the University of California at San Diego Medical Center, Department of Surgery, Division of Trauma, by the senior authors without regard to race, sex, ethnicity, or economic status. As a limited number of patients underwent BSA therapy in the time frame reviewed, all patients undergoing the procedure were included unless insufficient follow up information or documentation was available. Individuals undergoing the therapy all had chronic non-healing lower extremity wounds of ≥1 year duration that had not responded to traditional methods including a combination of off-loading orthopedic shoe, standard dressing applications, enzymatic debridement, skin substitutes and surgical debridement.

All patients underwent a complete history, physical examination, and imaging studies on initial presentation as is standard preoperative practice. The risks, benefits, complications, and alternatives to surgery were discussed with each individual prior to the procedure and an informed consent was attained. At the time of treatment, the patients were not enrolled in any study or receiving any experimental drug or device treatment. Although these data were not part of an active study and are retrospective in nature, Institutional Review Board approval was obtained to review charts for patients that had undergone BMA treatment. All identifying information was carefully withheld.

Any patient with gross clinical infection, including cellulitis or osteomyelitis, at the ulcer site, gangrenous changes, active Charcot neuroarthropathy, severe anemia, ankle/brachial index <0.6, serum albumin <2.5, renal failure with creatinine >2.5, malignancy of the lower extremity, or pregnant/nursing were excluded from BMA treatment as these factors are known to influence wound closure and may significantly reduce surgical benefits until addressed appropriately. With the exception of life-threatening situations, it is standard practice to control factors that may result in surgical failure, prior to performing any lower extremity procedure.

Evaluation and Procedure

The clinical chart of each patient was reviewed from the initial visit to the last available progress note. The age, body mass index, comorbidities, previous treatments/surgeries, wound etiology, and symptom onset date were recorded. Wounds were evaluated on the basis of size/surface area and underlying pathologic processes. Digital photographs were taken at the initial appointment and subsequent post surgical visits to monitor the progression of wound healing.

During a procedure where BMA is part of the treatment, basic surgical procedures are not altered. The patients are taken to the operating room and placed under general or monitored anesthesia care. An appropriate lower extremity block is also administered following counseling of the patient by the anesthesia team. The ulcers were surgically debrided to ensure a clean base with no eschar or fibrotic tissue. This allows direct contact of bone marrow cells to a viable wound tissue base. Through a lateral hindfoot approach, a trephine was utilized to harvest the marrow from the ipsilateral calcaneal bone. Approximately 3–5 cc of bone marrow aspirate was collected depending on the ulcer size. The aspirate was then immediately and directly applied to the wound bed so that the entire wound surface was coated with an even layer of the aspirate and then dressed with a equine or bovine xenograft, either Unite (Synovis, MN) or TissueMend (Stryker).

While all wounds were covered with a xenograft, at this time there is no evidence to suggest one xenograft product is superior to another except for perhaps qualitative differences such as cross linkage, which may affect the resistance of a product to proteases. Xenografts were chosen as the external wound explant or cover as they

may be left intact until wound closure. As all patients had wounds that were covered with a xenograft and historically data are available on use of xenografts, any differences from past data on xenografts alone, may be attributed to the addition of the BMA alone versus addition of the stem cell component with the BMA.

Figures 1–4 illustrate the wound pre- and post-debridement and BMA extraction, application, and coverage with xenograft of the BMA. The outer non-adherent and gauze dressings over the xenograft were kept intact, for approximately 7 days, or until their first postoperative visit, which was plus or minus 2 days. Patients were scheduled to be seen at the wound clinic weekly and had wound measurements for 12 weeks or until wound closure, whichever occurred first. The rate of wound improvement and/or failure was documented at each visit only if the xenograft was displaced and the wound visualized. If no significant decrease in wound size was recorded, of at least 0.5 cm after 6 weeks following surgery, then other therapies were considered to prevent further deterioration or infection.

RESULTS

The study consisted of 8 patients with lower extremity wounds secondary to past burns, vasculitic disease, and venous insufficiency, although secondary diagnosis included trauma, lupus, pyoderma gangrenosum, and/or lymphedema. The age, etiology and related co-morbidities, wound size, treatments utilized, and surgeries performed for each patient is listed in Table 1. Patients were placed on various local dressings before and after surgery as well if xenografts were displaced. Three of the 8 patients showed a gradual decrease in wound size over the following few months. One of the 3 patients had a left saphenous vein radiofrequency ablation per vascular recommendations 3 months following the BMA due to her significant varicosities and vascular disease. Two patients showed progressive increase in wound size several months following the procedure. The remaining 3 patients showed no significant improvement with less than 0.5 cm reduction in wound size after 6 weeks and therefore utilized alternative therapies. Two patients proceeded with the application

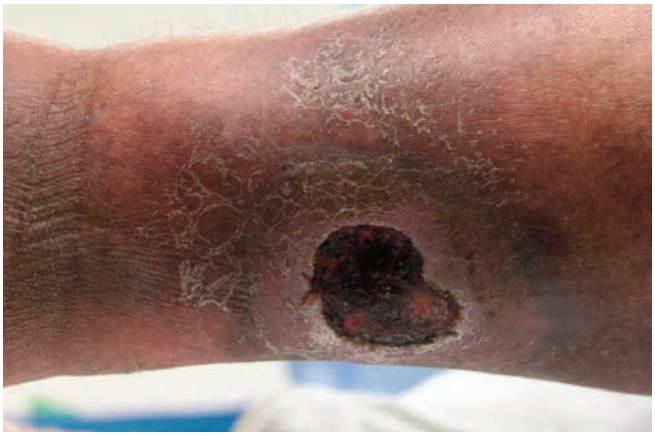

Figure 1.

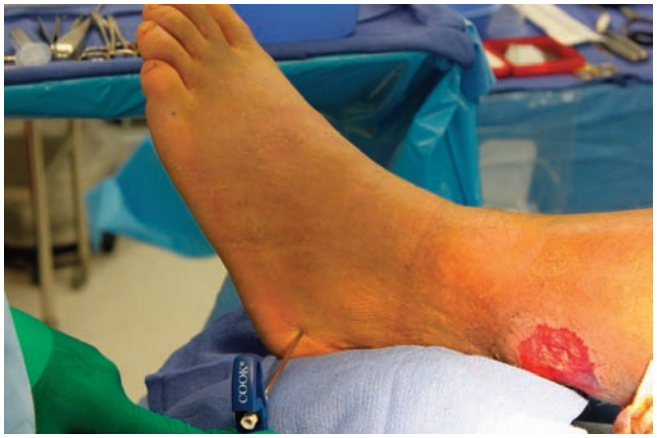

Figure 2.

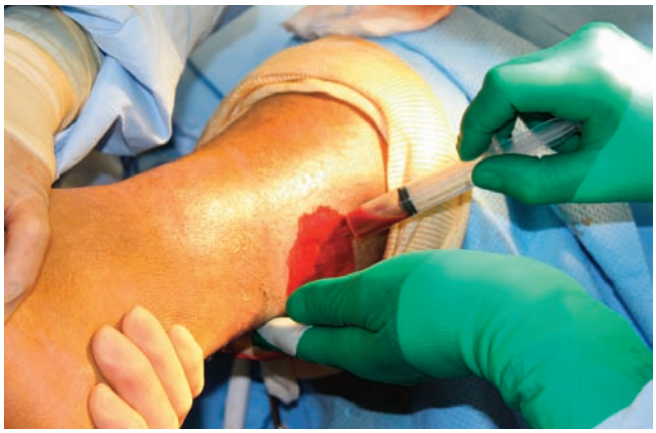

Figure 3.

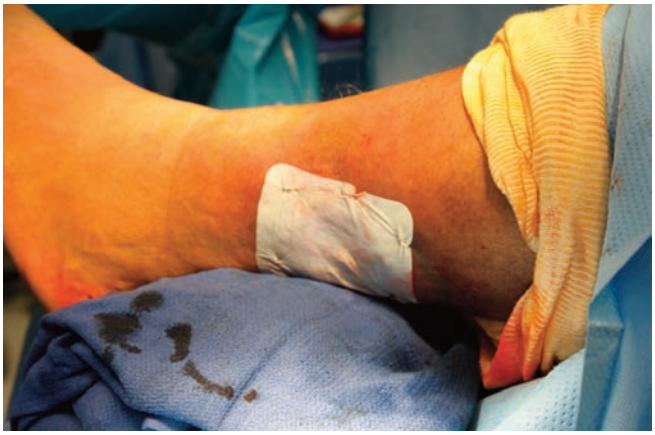

Figure 4.

Table 1

AGE	ETIOLOGY	WOUND	TREATMENT	SURGERY
32/M	Burn Injury, Trauma	**R LOWER LEG** 7/30/08 1.5x2x0.1cm 12/22/08 2.7x1.5x0.1cm **2/10/10 2.5x2x0.2cm** 3/31/10 2.2x1.7x0.1cm **4/21/10 2x1.3x0.1cm**	7/08: Panafil, Santyl 3/10: Fibracol, MediHoney Restore Silver	**2/18/10 Debridement, BMA & Xenograft™** 3/31: Dermagraft 4/21: Dermagraft
35/M	Venous Dz Lupus, RA Lymphedema	**L LATERAL LEG** 7/27/09 4.2x4.5x0.3 10/6/09 4.5x4.5x0.1 2/24/10 4.5x3.5x0.1 **3/3/10 4.2x3.5x0.1** **6/2/10 2.5x3.0x0.1**	7/09: Santyl Unna Boot 10/09: Silvadene, MediHoney 4/10: MeSalt 5/10: XCell	8/17/09: Xenograft™ 11/16/09: Xenograft™ 2/1/10: Demagraft 2/18/10: Apligraf **3/8/10 Debridement, BMA & Xenograft**
60/M	PVD Hep C Spondylarthrosis	**R LATERAL LEG** 2/10/09 0.8x1.0x0.1 6/24/09 1.0x1.0x0.1 11/30/09 2.0x2.2x0.1 **1/4/10 2.5x2.5x0.1** 3/11/10 3.0x2.5x0.1 4/19/10 3.5x2.5x0.1 5/25/10 2.5x2.2x0.1 **6/8/10 2.4x2.0x0.1**	2/09: Acticoat, Santyl 7/09: CoDa Study 10/09: Regranex, Silvadene 1/10: Acticoat 3/10: Fibracol, MediHoney 4/10: Prisma	6/9/09: Apligraf 6/24/09: Xenograft **1/11/10 Debridement, BMA & Xenograft** 4/5: Demargraft 4/12, 19: Apligraf 5/18, 25: Apligraf 6/1: Dermagraft
76/M	Trauma Hx R ankle ORIF s/p MVA Dermatitis	**L MEDIAL LEG** 8/13/08 3.1x2.1x0.1 2/11/09 0.2x0.2x0.1 5/26/09 1.6x1.5x0.1 **12/15/09 3.5x3.5x0.1** 2/10/10 3.4x2.6x0.1 4/5/10 4.3x5.0x0.1 5/12/10 5.1x4.4x0.1 **6/8/10 4.9x4.4x0.1**	8/08: Panafil, Restore Silver 12/08: Fibracol, Unna Boot, CoDa Study 11/09: Prisma 12/09: Fibracol 4/10: MediHoney 5/10: Fibracol	**1/11/10 Debridement, BMA & Xenograft™**
78/F	Venous Dz PAD	**L MEDIAL ANKLE** 1/27/09 0.8x1.0x0.1 3/10/09 2.5x2.0x0.1 11/18/09 1.8x1.8x0.1 **1/13/10 2.4x1.9x0.1** 3/3/10 1.5x1.5x0.1 4/14/10 1.5x1.4x0.1 6/1/10 1.5x1.1x0.1	4/09: CoDa Study 9/09: Azatreal 1/10: Fibracol 2/10: Xcell, Allevyn, Regranex 4/10: Mist Therapy	1/12, 2/10, 2/24, 3/10/09: Apligraf 10/20: MediHoney 11/18, 12/3: Apligraf **1/21/10 Debridement, BMA & Xenograft OA**
79/M	PG PVD	**R POSTERIOR LEG** 3/4/08 8.5x12.0x0.1 7/27/09: 3.5x2x0.1 1/26/10: 5.0x2.5x0.1, **2/10/10: 4.5x1.2x0.1,** 3/17/10: 3.5x2.5x0.1, **5/18: 6.7x4.3x0.5**	3/08: Aquacell Ag 2/10: Santyl 3/10: Acticoat, MediHoney, Unna Boot 5/10: Fibracol	1/08: Xenograft **2/18/10 Debridement, BMA & Xenograft™** 4/12-19: HBO 6/14: Xenograft 6/17: Debridement, STSG 6/18-22: HBO
92/F	PVD Varicosities	**L MEDIAL ANKLE** 1/27/10 2.5x1.5x0.1 **2/8/10 2.4x1.8x0.1** 3/31/10 1.5x1.5x0.1 4/21/10 2.1x1.3x0.1 5/11/10 2.4x0.7x0.1 **6 /7/10 1.6x1.0x0.1**	1/10: Santyl 2/10: Fibracol, Acticoat, Prisma	**2/24/10 Debridement, BMA & Xenograft™** 5/10: L RFA SSV
96/F	PVD PAD Trauma	**R LOWER LEG** 2/17/09 1.5x0.7x0.1 9/09 nearly closed 11/3/09 4.2x2.0x0.2 **1/6/10 6x2.5x0.1** 3/22/10 5.7x1.9x0.1 5/18/10 5.6x3.3x0.1 **6/9/10 6.1x3.6x0.2**	2/09: Acticoat 5/09: MediHoney 1/10: Mepilex 2/10: Acticoat 3/10: Fibracol, Regranex 5/10: Prisma	3/09: Dermagraft 10/15,27: Apligraf 11/3,12: Apligraf 12/1: Apligraf **2/4/10 Debridement, BMA & Xenograft** 5/10: Angiogram, angioplasy, stent R SFA

of living skin substitutes to aid in wound closure and one patient had split-thickness skin grafts placed over bilateral venous ulcers with the addition of hyperbaric oxygen therapy.

DISCUSSION

Autologous adult bone marrow-derived stem cells are known to assist with the tissue repair process by secreting large amounts of growth factors and cytokines. They are capable of differentiating into multiple cell types including endothelium, liver, muscle, skin, bone, cartilage, brain, fibroblasts and keratinocytes (20). Deng et al showed that the fluorescent labeled mesenchymal cells in mice gave rise to stem cells in the skin (21). In 2008, Rogers et al injected bone marrow aspirate topically into the wound periphery in 3 patients with differing etiologies and suggested this procedure as useful and safe adjunct to wound closure. These ulcers healed in 47, 50, 60 days respectively (22).

Similar results were achieved by Badiavas et al in which the 3 patients had complete closure of their yearlong ulcers with use of bone marrow aspirate and cultured cells. All healed within 3 months however 1 patient required a bioengineered skin (Apligraf) (23). In 2007, he conducted another study injecting BMA into the wounds of 4 subjects but only one healed completely (24). Our patient review showed wound size reduction in only 3 of the 8 patients without any patient's attaining closure in the 12 weeks post-operative period. Patients were routinely followed for their wounds for up to 6 months. Given the non-healing nature of these wounds, other products or procedures were utilized after 6 weeks when no improvement or deterioration was seen, to augment the healing process. At 6, 11, and 18 weeks after the application of BMA, 2 patients received living skin substitutes since the wounds appeared to have little to no intrinsic cell activity. Although there is strong evidence that mesenchymal stem cells can assist in wound healing, there are insufficient human studies with adequate number of subjects to prove the validity and efficacy.

The largest study to date using bone marrow-derived mesenchymal stem cells with or without autologous skin graft was published by Yoshikawa et al in 2008, which included 20 subjects with various nonhealing wounds (25). The authors reported complete healing in 18 patients and showed regeneration of native tissue by histologic examination. The study supported previous literature that bone marrow-derived stem cells are associated with dermal rebuilding, remodeling, increase in wound vascularity, and reduced fibrosis (25).

Falanga et al applied up to 3 applications of autologous culture-expanded mesenchymal stem cells with a fibrin glue system to acute wounds and chronic wounds. The acute wounds secondary to excision of non-melanoma skin cancers healed within 8 weeks. The chronic year-long lower extremity wounds significantly decreased or healed in 16 to 20 weeks, however this healing time does not appear to offer an advantage over healing times of other treatments published in the literature. This study showed a strong correlation between the number of mesenchymal stem cells per square centimeter surface area and reduction in ulcer size. The fibrin glue potentially keeps stem cells in the wound base and migrates out gradually as healing progresses (26). Injection of fibrin glue with adipose tissue-derived mesenchymal stem cells into the fistula tract in 25 patients showed healing rate of 71% with recurrence rate of 17.6% (27).

The present results suggest that fresh autologous bone marrow aspirate applied topically may help stimulate healing, but may not necessary lead to a significant visible decrease in wound closure within a given time frame compared to other treatment modalities that are currently approved and on the US and global market.

CONCLUSION

The authors are acutely aware of the many limitations of this review. The purpose of this small retrospective study was to look at a technique for stem cell extraction and determine any proof of concept. We acknowledge that this was not possible as the patients that had complete documentation and follow up were very limited. Furthermore, as the treated patients were not part of any on-going trial at the time of treatment, two different xenograft products were used. Ulcer etiologies and patient medical histories were also not demographically equal, making any conclusion about end results difficult. Two different types of xenograft were used. The two products differ in characteristics, which could have varying effects on outcomes, as non-cross linked products tend to rapidly degenerate and may be less effective in wounds with high levels of inflammatory enzymes.

We do believe that the review provides the reader with information on a new technique that warrants further investigation in a larger randomized controlled trial with demographically equal patients having the same wound etiology. We hope to initiate such a study in the near future. Finally, previously published data on xenografts alone has suggested that correctly applied flexible cross linked xenografts are ideal for problematic wounds, even without stem cells, in assisting with the closure of complex wounds.

REFERENCES

1. Bern H, Sheehan P, Boulton AJ. Protocol for treatment of diabetic foot ulcer. Am J Surg 2004;187:1S-10.
2. Phillips TJ. Chronic cutaneous ulcers: etiology and epidemiology. J Invest Dermatol 1994;102:38S–41.
3. Abbade LP, Lastoria S, De Almeida Rollo H, et al. A sociodemographic,clinical study of patients with venous ulcer. Int J Dermatol 2005;44:989.
4. CallamMJ, Ruckley CV, Harper DR,Dale JJ. Chronic ulcerationof the leg: extent of the problem and provision of care. Br Med J (Clin Res Ed) 1985;290:1855-6.
5. Morrell CJ, Walters SJ, Dixon S, Collins KA, Brereton LM, Peters J, Brooker CG. Cost effectiveness of community leg ulcer clinics: randomized controlled trial. BMJ 1998;316:1487–91.
6. Trent JT, Falabella A, Eaglstein WH, et al. Venous ulcers: pathophysiology and treatment options. Ostomy Wound Man 2005;5:38.
7. Bianco P, Riminucci M, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells: nature biology and potential applications. Stem Cells 2001;19:180-92.
8. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:31-49.
9. Krause DS, Theise ND, Collector MI, et al. Multiorgan, multilineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105:369-77.
10. Pittenger Mackay AM, Beck SC, et al. Multi-lineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
11. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N. From the cover: effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 2000;97:11307-12.
12. Watt FM, Hogan BL. Out of Eden: stem cells and their nitches. Science 2000;287:1427-30.
13. Stanley A, Osler T. Senescence and the healing rates of venous ulcers. J Vasc Surg 2001;33:1206-11.
14. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71–4.
15. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol 2001;69:513-21.
16. Lingen MW. Role of leukocytes and endothelial cells in the development of angiogenesis in inflammation and wound healing. Arch Pathol Lab Med 2001;125:67-71.
17. Mendez MV, Stanley A, Phillips T, Murphy M, Menzoian JO, Park HY. Fibroblasts cultured from distal lower extremities in patients with venous reflux display cellular characteristics of senescence. J Vasc Surg 1998;28;1040-50.
18. Raffetto JD, Mendez MV, Phillips TJ, Park HY, Menzoian JO. The effect of passage number on fibroblast cellular senescence in patients with chronic venous insufficiency with and without ulcer. Am J Surg 1999;178:107-12.
19. Vande Berg JS, Rudolf R, Holland C, Haywood-Reid PL. Fibroblast senescence in pressure ulcers. Wound Repair Reg 1998;6:38-49.
20. McFarlin K, Gao X, Liu YB, Dulchavsky DS, Kwon D, Arbab AS, Bansal M, Li Y, Chopp M, Dulchavsy SA, Guatam SC. Bone marrow-derived mesenchymal stromal cells accelerate wound healing in the rat. Wound Repair Reg 2006;14:471-8.
21. Deng W, Han Q, Liao L, Li C, Ge W, Zhao Z, You S, Deng H, Murad F, Zhao RC. Engrafted bone marrow-derived flk-(1þ) mesenchymal stem cells regenerate skin tissue. Tissue Eng 2005;11:110-9.
22. Rogers LC, Bevilacqua NJ, Armstrong DG.The use of marrow-derived stem cells to accelerate healing in chronic wounds. Int Wound J 2008;5:20-5.
23. Badiavas EV, Falanga V.Treatment of chronic wounds with bone marrow-derived cells. Arch Dermatol 2003;139:510-6.
24. Badiavas EV, Ford D, Liu P, Kouttab N, Morgan J, Richards A, Maizel A. Long-term bone marrow culture and its clinical potential in chronic wound healing. Wound Repair Regen 2007;15:856-65.
25. Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakura Y, Okuchi K, Nonomura A. Wound therapy by marrow mesenchymal cell transplantation. Plast Reconstr Surg 2008;121:860-77.
26. Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, Shrayer D,Carson P Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 2007;13: 1299-312.
27. Garcia-Olmo D, Herreros D, Pascual I, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: A phase II clinical trial. Dis Colon Rectum 2009;52:79-86.